Available online at www.sciencedirect.com

Journal of Ethnopharmacology 84 (2003) 17–21

www.elsevier.com/locate/jethpharm

Anti-inflammatory and analgesic properties of the stem bark extract of *Mitragyna ciliata* (Rubiaceae) Aubrév. & Pellegr.

A.B. Dongmo[a,*], A. Kamanyi[b], G. Dzikouk[c], B. Chungag-Anye Nkeh[d], P.V. Tan[b], T. Nguelefack[b], T. Nole[d], M. Bopelet[e], H. Wagner[e]

[a] *Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon*
[b] *Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon*
[c] *ENS P.O. Box: 47 Yaoundé, University of Yaoundé I, Cameroon*
[d] *Faculty of Science, P.O. Box 812, University of Yaoundé I, Cameroon*
[e] *Institute of Pharmaceutical Biology, Butenandstr. 5–13, Munich, Germany*

Accepted 22 August 2002

Abstract

Mitragyna ciliata is widely used in traditional medicine for the treatment of inflammation, hypertension, headache, rheumatism, gonorrhoea and broncho-pulmonary diseases. In the present study, the anti-inflammatory and analgesic properties of the stem bark extract of *M. ciliata* were investigated. The stem bark of this plant was extracted over Soxhlet with hexane followed by another extraction with methanol. The resulting methanol extract was used for the pharmacological test. Anti-inflammatory activity was evaluated on the basis of the inhibitory effect of the extract on 5-lipoxygenase, and carrageenin-induced hind paw oedema in the rat. The methanol extract, at a dose of 19.2 µg/ml, exhibited no inhibition on 5-lipoxygenase. However, this extract administered per os (50 mg/kg) produced about 70% inhibition of carrageenin-induced paw oedema 1 h after administration. This inhibition was maintained to about 50% 2 h after administration. The dose of 50 mg/kg of MeOH extract significantly decreased sensitivity to pain from 78.75 to 107.5 g These findings suggest that extracts of the bark of *M. ciliata*, possess potent anti-inflammatory and analgesic effects. Chemical analysis of the extract showed the presence of alkaloids and kaempferol derivative which may be responsible for the anti-inflammatory properties.

© 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Anti-inflammatory; Analgesic; 5-Lipoxygenase; Carrageenin

1. Introduction

A scientific evaluation of plants and the traditional methods of their use in disease management can permit their incorporation into the official health care systems in Africa and in developing countries elsewhere. The stem bark of *Mitragyna ciliata* (Rubiaceae), Aubrev. & Pellegr, is generally used in traditional medicine in West Africa to cure fever (Halle, 1966), manage hypertension, and to facilitate delivery (Bouquet and Debray, 1974). The decoction of the stem bark is also used for the treatment of broncho-pulmonary diseases, dysentery, rheumatism and gonorrhoea (Bouquet, 1969). The genus *Mitragyna* is very rich in alkaloids and many have been isolated from the leaves, stem bark, and root-bark. The major alkaloids are mitraphylline (2-methoxy corynantheine), rotundifoline, rhynchophyline, isorotundifoline, ciliaphyline, rhynchociline, mitragynine and speciociliatine (Shellard, 1983; Duke, 1992a,b). Some of these alkaloids, particularly mitraphylline and mitragynine, possess a local anaesthetic activity, induce a fall of blood pressure, and increase cardiac frequency (Bouquet and Debray, 1974). Although, *M. ciliata* is widely used in ethnomedicine, its anti-inflammatory properties have not yet been pharmacogically evaluated. In order to understand the pharmacological basis for the use of *M. ciliata* in folk medicine for the treatment of inflammation illnesses, the present study was designed

---

[*] Corresponding author. Tel.: +237-777-4003; fax: +237-337-0424
E-mail address: adongmo@hotmail.com (A.B. Dongmo).

0378-8741/02/$ - see front matter © 2002 Elsevier Science Ireland Ltd. All rights reserved.
PII: S0378-8741(02)00252-0

to investigate the effect of the methanol extract on 5-lipoxygenase enzyme and carrageenin-induced-paw oedema in the rat, as well as analgesic activity.

2. Material and methods

2.1. Plant material

M. ciliata was collected around the Yaounde city in 1997. Voucher specimen No.HNC/60750 was deposited at the National Herbarium, Yaounde. The dried bark of *M. ciliata* was reduced to a very fine powder and 200 g Soxhlet-extracted, respectively, with hexane for 48 h to eliminate lipophilic compounds and with MeOH for 24 h. The resulting MeOH extract solution was concentrated in vacuum using a rotavapor to obtain a brown powder (20 g). In order to identify the different chemical groups present, the extract was chromatographed on TLC and the presence of cumarine, herzglycosids and saponins was tested according to Wagner and Bladt (1996). The chromatographed extract was compared with rutin, chlorogenic acid and kampferol derivatives, quercetin and isoquercetin, as reference substances. A mixture of ethyl acetate/formic acid/glacial acetic acid/water (100:11:11:26) was used as the mobile phase. The silica gel plate was first observed under UV_{254nm} and UV_{366nm}, and then sprayed with natural products reagent (NP/PEG). TLC (silica gel) was performed on the methanol extract using Toluene/EtOAc/diethylamine (70:20:10) as the mobile phase. Alkaloids were detected by modified Dragendorff reagent as orange-brown zones which appeared immediately on spraying.

3. Pharmacological studies

3.1. Anti-inflammatory activity

3.1.1. 5-lipoxygenase assay

The inhibitory effect of the *M. ciliata* extract on 5-lipoxygenase activity was measured by the reduced production of 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) from [^{14}C]arachidonic acid (Wagner and Fessler, 1986) in comparison with a blank run without inhibitor. Porcine leukocytes, isolated from fresh peripheral blood by dextran sedimentation and centrifugation, were resuspended in phosphate buffered saline (15×10^7 cells/ml). A total of 2.5 ml of the suspension were incubated at 37 °C with 50 μl extract dissolved in ethanol or with 50 μl pure ethanol as the control. The leukocytes were challenged by addition of 15 μM of the calcium ionophore A23187 and 2 mM $CaCl_2$ in the presence of 0.1 μCi 1-[^{14}C]arachidonic acid and 10 μM eicosatetraenoic acid in order to suppress the arachidonic acid metabolism via 12-lipoxygenase in favour of 5-lipoxygenase (in modification of the method of Kuhl et al., 1984). After 5 min, 0.5 ml of 1% formic acid was added to stop the reaction. Arachidonic acid and its metabolite were extracted with ethyl acetate, separated by reverse-phase HPLC (acetonitrile/water containing 0.1% 1 N H_2PO_4, gradient elution: 50–90% acetonitrile in 20 min, 10 min 90% acetonitrile isocratic elution) and detected by radioactivity- monotoring. The results were expressed as means of percentages of three experiments (% inhibition). The rate of inhibition in % is calculated as follows:

$$\text{Inhibition (\%)} = \frac{100 - (\text{5-HETE with inhibitor}) \times 100}{(\text{5-HETE without inhibitor})}$$

3.2. Carrageenin-induced paw oedema

Anti-inflammatory activity was evaluated on the basis of the inhibition of the carrageenin-induced hind paw oedema (Winter et al., 1962). The extract and water were given 30 min to the experimental and control groups respectively before administration of the oedema-inducing agent (0.1 ml of a 1% carrageenin suspension in 0.9% NaCl), which was injected into the plantar surface of the left hind paw of male and female Wistar rats weighing (150–180 g). The volumes of the injected paws were measured 30, 60, 120 and 240 min after induction of inflammation using an Ugo Basile Plethysmometer No 7140. Oedema was expressed as the increase in paw volume due to carrageenin injection. Reference groups were treated with acetylsalicylic acid (20 mg/kg). The extract and the reference drugs were dissolved in 2% dimethylsulphoxide just before use.

3.3. Analgesic activity

Analgesic activity of the extract of *M. ciliata* was tested in male and female Wistar rats (150–175 g) using the Ugo Basile analgesimeter No. 7200. In these experiments, force was applied to the left hind paw of the experimental animals using an analgesy-meter which exerts a constantly increasing force on the rat paw. The rat was suspended vertically while its left hind paw was placed between the plinth and the finger. As the applied force increases, it gets to a point where the animal struggles to free its paw. This is the level at which the animal feels pain. The weight causing pain before treatment and 2 h after treatment of the animals with the extract and the various test drugs was determined.

3.4. Statistical analysis

Each results represents the mean value ± S.E.M. The comparison of mean values was done using the Student-

t-test. A *P* value less than 0.05 was considered as significant and *P* less than 0.01 being very significant.

4. Results

4.1. Chemical analysis

Chemical analysis carried out on the plant extract with comparison to known compounds indicated the presence of kaempferol derivative and alkaloids. After spraying the TLC plate by modified Dragendorff reagent, many orange-brown zones were observed, indicating the presence of a number of alkaloids. The presence of cumarine, herzglycosids and saponins were not found.

4.2. Anti-inflammatory effect

The MeOH extract of *M. ciliata* showed no inhibitory effect on 5-lipoxygenase activity up to a dose of 19.2 µg/ml (Table 1). Table 2 shows the inhibitory effect of the extract and reference compounds on carrageenin-induced oedema. For each of the two doses of extract tested (50–100 mg/kg), the MeOH extract showed a maximum anti-oedematous effect 1 h after carrageenin administration, with about 70% inhibition of oedema development for the 50 mg/kg dose. This inhibitory effect was significantly maintained at about 50% 2 h after administration of the oedematous agent, followed by a significant drop to 26–28% inhibition 2 h later. The anti-oedematous effect induced by the reference compounds progressively increased up to 2 h for aspirin (60% inhibition) following carrageenin administration. Table 3 shows that the extract at a dose of 100 mg/kg significantly increased the analgesimeter-induced force at which pain was felt from 67 g in the controls to 107 g at the dose of 50 mg/kg. Acetylsalicylic acid (20 mg/kg) and indomethacin (7 mg/kg) were equally effective as the extract of *M. ciliata* but the ability of indomethacin to reduce sensitivity to pain was double that observed for acetylsalicylic acid at the doses tested.

Table 1
Inhibition of 5-lipoxygenase by extracts of *M. ciliata*

Extracts	Concentration (µg/ml)	% Inhibition
Hexane extract	19.2	3.60
	38.4	12.60
MeOH extract	19.2	0.00

$n = 3$.

5. Discussion

The MeOH extract of *M. ciliata* exhibited no inhibitory effect on 5-lipoxygenase at a dose of 19.2 µg/ml. The absence of an inhibitory effect on 5-lipoxygenase does not necessarily indicate a lack of anti-inflammatory activity. The active compound(s) present in the extract may act at other sites implicated in the inflammatory process, since the extract reduced carrageenin-induced paw oedema in rat, indicating an anti-inflammatory effect. The anti-oedematous response induced by the extract was significant during the first phase of oedema development, suggesting an inhibitory effect on the release of active pain substances such as histamine, serotonin or polypeptides. This anti-oedematous response was significantly maintained during the second and third phases of oedema development, suggesting an inhibition of the release of kinins or cycloxygenase, one of the enzymse involved in the formation of prostaglandins that induced the inflammation process (DiRosa et al., 1971). Another important action of mild anti-inflammatory products, e.g. salicylates, is the reduction of the increased capillary permeability that occurs in inflammation. In the present study, the time course anti-inflammatory trend of results for the reference drugs are similar to those reported by other workers (Chagnon et al., 1983; Claeson et al., 1993). The anti-oedematous response was significantly

Table 2
Anti-inflammatory activity of the methanol extract of *M. ciliata*, indomethacin and acetylsalicylic acid on rat hind-paw oedema induced by carrageenin

Treatment	ΔV (ml)				%			
	30 min	60 min	120 min	240 min	30 min	60 min	120 min	240 min
Control	1.05 ± 0.16	1.32 ± 0.13	1.90 ± 0.36	2.30 ± 0.20				
Acetylsalicylic acid	0.85 ± 0.16	0.85 ± 0.21	$1.00 \pm 0.28^{*}$	$1.05 \pm 0.31^{**}$	28.57	58.33	60.00	60.00
M. ciliata extract (50 mg/ml)	$0.68 \pm 0.13^{*}$	$0.69 \pm 0.23^{*}$	$0.63 \pm 0.30^{*}$	0.90 ± 0.15	35.24	69.69	52.63	28.26
M. ciliata extract (100 mg/ml)	$0.51 \pm 0.02^{**}$	$0.46 \pm 0.06^{**}$	$1.00 \pm 0.15^{*}$	$1.70 \pm 0.11^{*}$	51.43	65.15	47.36	26.08

Oedema is expressed as increase in paw volume (ΔV) ± S.E.M. $n = 5$% values refer to % reduction in paw volume compared to control, at each time point.

* $P < 0.05$: statically significant relative to control.

** $P < 0.01$: statically significant relative to control.

Table 3
Analgesic effects of the methanol extract of *M. ciliata* in rats

Drugs used	Dose (mg/kg)	Weight causing pain (g) ± S.E.M.	
		Before administration	Two hours after administration
Control	0	67.85 ± 7.39	67.50 ± 5.98
Acetylsalicylic acid	20	56.25 ± 4.98	88.75 ± 8.33
Indomethacin	7	77.50 ± 7.68	101.25 ± 14.26*
M. ciliata (MeOH extract)	100	68.53 ± 6.82	100.20 ± 7.07*
	50	78.75 ± 10.48	107.50 ± 8.31*

Mean ± S.E.M. $n = 5$.
* $P < 0.05$: statically significant relative to control.

lower in rats pre-treated with indomethacin than in rats pre-treated with acetylsalicylic acid. However, the action of these reference substances was longer compared with that of the extract, whose activity peaked 60 min after carrageenin administration and then progressively declined to below initial (30 min) values 3 h later. These differences in onset time and intensity of anti-oedematous effect between the MeOH extract and the reference compounds could be due to their pharmacokinetic and pharmacodynamic properties (McGaw et al., 1997). Such pharmacokinetic differences are usually related to differences in first pass metabolism as well as the rate of hepatic degradation.

The MeOH extract of *M. ciliata* also exhibited potent analgesic activity by significantly increasing the threshold of sensitivity to pain in the rats. The analgesic effect of the extract may be due to its anti-inflammatory action as is the case with salicylates, which are particularly effective in relieving the type of pain associated with inflammation or oedema (Passmore and Robson, 1970). The analgesic effect of the plant extract might be explained also by the depletion of the substance P from primary sensory neurons as a consequence of the reduced production of prostaglandin (Yaksh et al., 1979).

The chemical analysis of the plant extract using different methods described indicated the presence of kaempferol derivative and alkaloids, which might be in part responsible for these pharmacological effects (Matsumoto et al., 1996).

The present results support the ethno-medical application of *M. ciliata* in the treatment of inflammation diseases. Further experimentation is needed in order to understand the precise mechanism of action in anti-inflammatory activities by the extract.

Acknowledgements

The financial assistance of the DAAD (Deutscher Akademischer Austauschdient) accorded to three of the authors for their research mission in Germany at the Institute for Pharmaceutical Biology of Prof H.Wagner in Munich, where a good part of this work was carried out, is hereby acknowledged.

References

Bouquet, A., 1969. Féticheurs et médecines traditionnelles du Congo (Brazzaville). ORSTOM, Paris, p. 221.

Bouquet, A., Debray, M., 1974. Plantes médicinales de la Côte d'Ivoire. ORSTOM, Paris, pp. 148–149.

Chagnon, M., Ndibwami, A., Dubé, S., Bumaya, A., 1983. Activité anti-inflammatoire d'extraits de *Crassophalum multicorymbosum*. Planta Medica 49, 255–256.

Claeson, P., Panthong, A., Tuchinda, P., Reutrakul, V., Kanjanapothi, D., Taylor, W.C., Santisuk, T., 1993. Three non phenolic diarylheptanoids with anti- inflammatory activity from *Curcuma xanthorrhiza*. Planta Medica 59, 451–454.

DiRosa, M., Giroud, J.P., Willoughby, A., 1971. Studies of the mediators of the acute inflammatory response induced in rats different sites by carrageenin and turpentine. Journal of Pathology 104, 15–20.

Duke, J.A., 1992a. Handbook of Biologically Active Phytochemicals and their Activities. CRC Press, Boca Raton, FL.

Duke, J.A., 1992b. Handbook of Phytochemical Constituents of GRAS Herb and other Economic Plants. CRC Press, Boca Raton, FL.

Halle, N., 1966. Famille des Rubiacées 1ère partie. Flore du Gabon, Muséum national d'histoire naturelle, Paris.

Kuhl, P., Shiloh, R., Jha, H., Murawski, U., Zilliken, F., 1984. Human and Porcine 6,7,4′-trihydroxyisoflavan: a potent and selective inhibitor of peripheral blood leukocytes. Prostaglandins 28, 783–805.

Matsumoto, K., Mizowaki, M., Schitra, T., Murakami, Y., Takayama, H., Sakai, S., Aimi, N., Watanabe, H., 1996. central antinociceptive effects of mitragynine in mice: contribution of descending noradrenergic and serotonergic systems. European Journal Pharmacology 317, 75–81.

McGaw, L.J., Jäger, A.K., Van staden, J., 1997. Prostaglandin synthesis inhibitory activity in zulu, Xhosa and Sotho medicinal plants. Phytoterapy Research 11, 113–117.

Passmore, R., Robson, J.S., 1970. A comparison to medical studies. In: Pharmacology, Microbiology, General pathology and Related Subjects, vol. 2. Blackwell Scientific Publications, Oxford, pp. 546–555.

Shellard, J.E., 1983. Mitragyna: a note on the alkaloids of African species. Journal of Ethnopharmacology 8, 345–347.

Wagner, H., Bladt, S., 1996. Plant Drug analysis. Springer Verlag, Berlin, pp. 258–261.

Wagner, H., Fessler, B., 1986. In-vitro 5-lipoxygenasehemmung durch *Elipta alba* extracte und das coumestan-derivaz Wedelolacton. Planta Medica 52, 374–377.

Winter, C.A., Risley, E.A., Nuss, W.G., 1962. Carrageenin-induced edema in hind paw of the rats as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine 111, 544–547.

Yaksh, T.L., Farb, D.H., Leemann, S.E., Jesell, Th.M., 1979. Inthrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. Science 206, 481–485.